CORVINUS
UNIVERSITY of
BUDAPEST

Faculty of Food Science

Book of proceedings

FOOD SCIENCE CONFERENCE 2013

- With research for the success of Darányi Program

7-8th November, 2013.
Budapest
Hungary

EFFECTS OF DIFFERENT SUBSTRATES ON GROWTH OF *SHEWANELLA XIAMENENSIS*

Attila Szöllősi - Nándor Bárány - Ágoston Hoschke - Judit M. Rezessy-Szabó - Quang D. Nguyen

[1]Corvinus University of Budapest, Faculty of Foods Science, Department of Brewing and Distilling
H-1118 Budapest Ménesi str. 45, attila.szollosi@uni-corvinus.hu

SUMMARY

Shewanella xiamenensis is known as iron-reducing bacteria, thus it has potential on application in microbial fuel cell systems. In this study, effects of different carbohydrates (glucose, fructose, galactose, maltose, lactose, saccharose and starch) and organic acids (citric, tannic, lactic and tartaric) on utilization and growth of bacteria were investigated using the optical density change and HPLC methods. The results show that S. xiamenensis grew rapidly on monosaccharide (glucose, fructose, galactose) and disaccharides (maltose and saccharose) carbon sources. In the case of starch and lactose the lag-phase was longer that the cases mentioned above. Bacteria were also able to growth on all investigated organic acids, except tartaric acid, with longer lag-phrase. These results will support technological development of MFC using Shewanella culture.

1. INTRODUCTION

Developing carbon-neutral renewable energy sources is an important research area for alternative power systems (Biffinger, *et al.*, 2012). The production of electricity from different wastewaters would be soon an economically desirable process because of the worldwide population increase and energy supply exhaustion (Hashemi and Samimi, 2012).
In a conventional biofuel process, the chemical energy contained in biomass and organic waste can be recovered in different forms (bio-ethanol, bio-methanol, bio-hydrogen, biogas, *etc.*). To use the conserved energy of the carrier molecules, more step(s) are needed to release the energy (*e.g.* burning) that occur relevant losses. Bioelectricity could be directly generated from wastewater using a microbial fuel cell (MFC). The overall conversion efficiencies of MFCs that can be reached are potentially higher compared to other biofuel processes (Rabaey, *et al.*, 2005).

Shewanella xiamenensis is a marine microbe and firstly isolated from coastal sediment (Xiamen, China). The physiological and biochemical features of this species were similar to those of members of the genus *Shewanella*, and it was most closely related to *S. oneidensis* (Huang, et al., 2010) that now became a frequent-used alternative power microbe in marine applications (Gorby, et al., 2009). Meanwhile many scientific data are available in literature about this organism, so far very few studies are published relating to S. xiamenensis.

In this study, the metabolism of different carbohydrates and organic acids of *Shewanella xiamenensis* are focused.

2. MATERIALS AND METHODS

2.1. Culture and maintenance

Shewanella xiamenensis strain DSMZ 22215 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and applied as exo-electron producer. Marine agar and Luria-Bertrani (LB) broth were used for inoculation of bacteria at 30°C.

2.2. Media and growth

Different mono-, (D-glucose, D-fructose, D-galactose,) di- (D-maltose, D-lactose and D-sucrose) and polysaccharides (starch) as well as organic acids (citric, tannic, lactic and tartaric) were supplemented in concentration of 2 $g \cdot L^{-1}$ to growth medium (Marine broth) that consists of 5.84 $g \cdot L^{-1} NaCl$, 0.1 $g \cdot L^{-1}$ KCl, 0.25 $g \cdot L^{-1}$ NH_4Cl, 12 $g \cdot L^{-1} Na_2HPO_4 \cdot 12H_2O$, 2.57 $g \cdot L^{-1}$ $NaH_2PO_4 \cdot 2H_2O$. The growth of the microorganism was followed by monitoring changes of optical density at wavelength of 600 nm (OD600nm) with spectrophotometer.

2.3. Determination of sugars and organic acids

The respective saccharides and organic acids in the sample solution were determined using Thermo Scientific Corporation Surveyor HPLC system. The system consists of quadruped Surveyor pump, Surveyor automatic injector, Surveyor RI and PDA 210 detector and Aminex HPX-87H column (Bio-Rad). Parameters of measurement: time of running was 25 minutes; eluent was 0,005 N sulphuric acid; volume of injection (10 µl) depended of carbohydrate-concentration of samples; the temperature of the column and detector was 45 °C; the flow-rate was 0.5 ml/min.

3. RESULTS AND DISCUSSION

Shewanella xiamenensis strain DSMZ 22215 was able to utilise and grow on wild range of carbon sources (Figure 1). All investigated monosaccharaides (glucose, fructose and galactose) were utilized properly by bacterium. In these cases, lag-phases were short and relevant quantity of cell mass was produced. The microbe also utilized the disaccharides (maltose, lactose and sucrose) very efficiently. Sucrose and maltose were metabolized faster, and in the case of lactose the lag-phase was longer. This result is in agreement with one published by Nagy (2002) and it can be explained that rate of synthesis of □-galactosidase by *S. xiamenensis* cells was low in the initial phase. The growth of bacteria was turned to exponential phase when the level of □-galactosidase was as high as enough to hydrolyze lactose to glucose and maltose. The profile of growth curve (OD600nm) of bacterium in the case of starch as substrate was very similar to lactose case. Starch was quite as complex substrate as to easily to be utilized by bacterium cells. Moreover, in this case the specific growth-rate significantly lower than the case of lactose as substrate

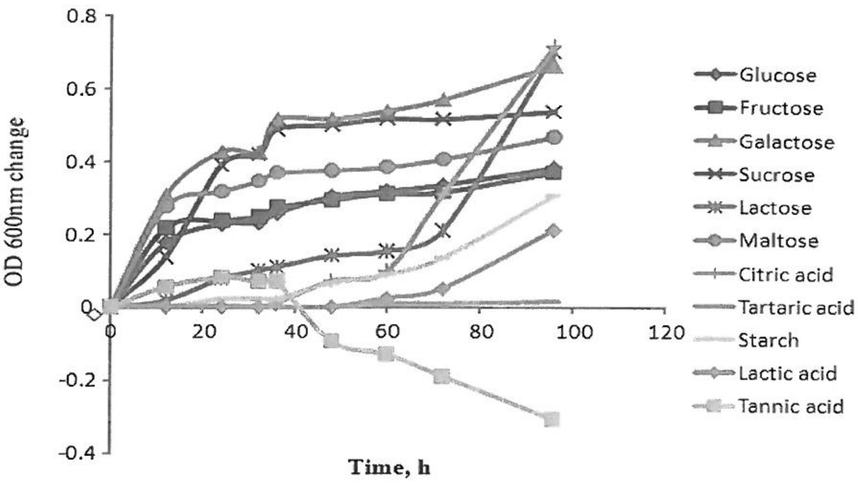

Figure1: OD_{600} change during the experiment

The organic acids were also metabolized by *S. xiamenensis*, except of tartaric acid. Comparing with saccharides generally the long lag-phase and low biomass production was detected. However, citric acid seemed to be good carbon source for production of biomass. Lactic and tannic acid were also metabolized, but they served less effective energy source than the citric acid. In the case of tannic acid the decrease in absorbance at 600nm was monitored meaning *S. xiamenensis* was unable to grow and utilize this substrate.

The consumption rates of individual substrate – calculated by HPLC – are illustrated on Fig. 2. In the cases of sucrose, lactose, galactose, fructose and glucose, the consumption rates were 0.142 g/24 hours, 0.137, 0.130, 0,113 and 0.067 g/24 hours, respectively.

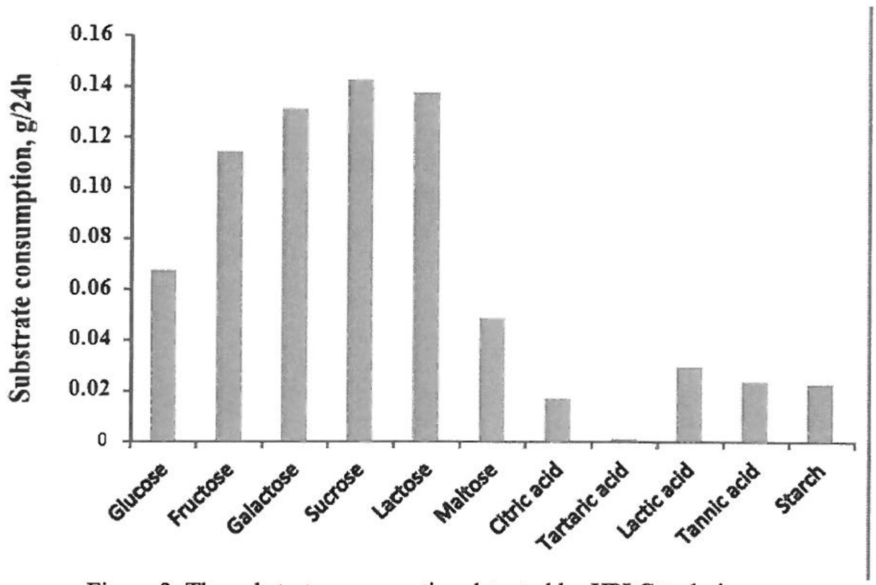

Figure 2: The substrate consumption detected by HPLC technique

The consumption rates of organic acids were definitely lower than the ones of mono- and disaccharides.

4. CONCLUSIONS

Shewanella xiamenensis was able to grow on media supplemented with several sugars and organic acids. Moreover, this species had utilized lactose, galactose, maltose and glucose at high consumption rate, thus it can be applied to develop technology for processing of food industry wastes.

ACKNOWLEDGEMENT: *This work is supported by National Development Agency (Project no. TÁMOP-4.2.1./B-09/1-KMR-2010-0005, TÁMOP-4.2.2/B-10/1-2010-0023 and TECH_09-A3-2009-0194), as well as Bolyai Research Grant from Hungarian Academic of Sciences.*

5. REFERENCES

Biffinger, J. C., Fitzgerald, L. A., Ray, R., Little, B. J., Lizewski, S. E., Petersen, E. R., Ringeisen, B. R., Sanders, W. C., Sheehan, P. E., Pietron, J. J., Baldwin, J. W., Nadeau, L. J., Johnson, G. R., Ribbens, M., Finkel, S. E., Nealson, K. H. (2012): The utility of Shewanella japonica for microbial fuel cells. Bioresource Technology, 102 (1), 290-297

Gorby, Y. A., Yanina, S., Mclean, J. S., Rosso, K. M., Moyles, D., Dohnalkova, A., Beveridge, T. J., Chang, I. S., Kim, B. H., Kim, K. S., Culley, D. E., Reed, S. B., Romine, M. F., Saffarini, D. A., Hill, E. A., Shi, L., Elias, D. A., Kennedy, D. W., Pinchuk, G., Watanabe, K., Ishii, S., Logan, B., Nealson, K. H., Fredrickson, J. K. (2009): Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms (vol 103, pg 11358, 2006). Proceedings of the National Academy of Sciences of the United States of America, 106 (23), 9535-9535

Hashemi, J., Samimi, A. (2012): Steady state electric power generation in up-flow microbial fuel cell using the estimated time span method for bacteria growth domestic wastewater. Biomass & Bioenergy, 45 65-76

Huang, J. X., Sun, B. L., Zhang, X. B. (2010): Shewanella xiamenensis sp nov., isolated from coastal sea sediment. International Journal of Systematic and Evolutionary Microbiology, 60 1585-1589

Nagy, Z. (2002): A penicillium chrysogenum laktóz hasznosításának vizsgálata, Mikrobiológiai és Biotechnológiai Tanszék, Debreceni Egyetem, Debrecen

Rabaey, K., Lissens, G., Verstraete, W. (2005): Microbial fuel cells: performances and perspectives IWA Publishing London, Seattle